25. (Amended) A method of producing glial cells from neural stem cells, the progeny of said neural stem cells being capable of differentiating into neurons, astrocytes, and oligodendrocytes, said method comprising the steps of:

(a) isolating said neural stem cells from a donor,

(b) proliferating the isolated neural stem cells in a first culture medium containing a growth factor to produce precursor cells, and

(c) differentiating the precursor cells in a second culture medium that is substantially free of said growth factor to obtain glial cells.

31. (Amended) A cell culture comprised of glial [Glial] cells derived from a clonally-derived neurosphere [formed by the method of Claim 25].

32. (Amended) A cell culture comprised of precursor cells derived from a clonally-derived neurosphere [in vitro living in a culture medium having a growth factor].

33. (Amended) [An] A cell culture comprised of oligodendrocytes derived from a clonally-derived neurosphere [precursor cell and living in a culture medium].

34. (Amended) [An] A cell culture comprised of astrocytes derived from a clonally-derived neurosphere [precursor cell and living in a culture medium].

Please cancel Claims 18-24, **without** prejudice or disclaimer, as being drawn to a non-elected invention.

REMARKS

The claims have been amended to more succinctly define the claimed subject matter.

Rejections under 35 U.S.C. § 101

Applicants note the provisional double patenting rejection of Claims 1-17 and 25-35. Applicants request deference in addressing this rejection until there is an indication of allowable subject matter.

Claims 1 to 17 stand rejected under § 101 for lack of evidence that the invention works as claimed. The enclosed declaration of Dr. Joseph P. Hammang demonstrates that, by using substantially the same methods as disclosed in the application (see Example 2 of specification), neurons can be remyelinated as claimed. Accordingly, it is believed that the rejection to Claims 1 to 17 is overcome.

Rejections under 35 U.S.C. § 112

The specification stands objected to and Claims 1-17 and 35 stand rejected under § 112, first paragraph. The Examiner states that applicants have failed to provide evidence in the form of experimental results indicating that the claimed methods give the claimed results. In view of the declaration of Dr. Hammang, it is believed that adequate supporting evidence has now been supplied and that this rejection is overcome.

With regard to Claim 1, the Examiner states that the term "associating" must "be limited to the type of injection and the site of injection...", with the assumption that the claims have been interpreted as being drawn to *in vivo* application. While the primary utility of the claimed invention is for *in vivo* applications, there are *in vitro* applications of the claimed method, adequately enabled by the disclosure, which would be apparent to those of ordinary skill in the art. Accordingly, Claim 1 has been amended to address the rejection under §112, second paragraph, in which the Examiner objects to the term "associating". It is believed that the term "causing the harvested precursor cells to come into contact with a demyelinated axon" more succinctly defines

the invention. It is believed that the Examiner probably intended to raise the same rejection with respect to Claim 7. Accordingly, that claim has also been amended in the same way.

Rejections under 35 U.S.C. § 102

Claims 25-28, and 30-34 stand rejected under § 102 (b) as being anticipated by Hunter *et al.* For a proper rejection under § 102 (b), every element of the claimed invention must be disclosed in the cited reference.

The Examiner characterizes Hunter *et al.* as disclosing production of glial cells from neural stem cells. However, the methods of Hunter *et al.* start with glial progenitor cells (see Abstract) rather than neural stem cells. A stem cell is an undifferentiated cell capable of proliferation, self-maintenance and the production of a large number of differentiated, functional progeny; see Potten and Loeffler, Development 110:1001-1020 (1990), attached as Appendix A. Also attached as Appendix B is review article on stem cells by Hall and Watt that illustrates these and other stem cell characteristics. While progenitor cells are capable of some proliferation, the number of divisions is limited before each of the cells differentiate. Consequently, the progenitor cells of Hunter et al are not capable of self-maintenance. Thus, a neural stem cell may divide to yield a daughter stem cell, and, for example, a daughter glial progenitor cell. The glial progenitor cell will have only a limited capacity for proliferation and will ultimately differentiate into an astrocyte or oligodendrocyte. The daughter stem cell, on the other hand, is capable of unlimited self-renewal and can have neuronal or glial progenitor progeny, that will ultimately differentiate into neurons or glial cells (astrocytes or oligodendrocytes).

Because Hunter *et al.* disclose methods of producing glial cells from progenitor cells rather than neural stem cells, as recited in part (a) of Claim 25, the reference does

not disclose every element of the claim and thus cannot be anticipatory under § 102 (b). However, in order to more succinctly describe the cells used in the method of Claim 25 to produce glial cells, the claim preamble has been amended to recite that glial cells are produced "from neural stem cells, the progeny of said neural stem cells being capable of differentiating into neurons, astrocytes, and oligodendrocytes". While Hunter *et al.* describe bipotential progenitor cells that can differentiate into type II astrocytes and oligodendrocytes, there is no disclosure of a culture of neural stem cells, with the capacity to exhibit self-renewal, generate large numbers of progeny, and produce progeny that can also differentiate into neurons in addition to type I and type II astrocytes and oligodendrocytes.

Regarding Claims 31-34, the Examiner states that "Hunter discloses the production of astrocytes, oligodendrocytes, precursor cells and glial cells...". Claims 31-34 have been amended to further define that the claimed cells are derived from a clonally-derived neurosphere. The clonal nature of the cells of a neurosphere is discussed on p. 9, line 30 to p. 10, line 24, wherein it is disclosed that individual cells are cultured according to the disclosed methods and form neurospheres. Thus, the cell cultures of Claims 31-34 are distinguishable from those described by Hunter *et al.* which are not clonally-derived. With the clonally-derived cells of a neurosphere, it is known that all of the cells present in the neurosphere were proliferated in the culture and were obtained from a single cell.

Rejections under 35 U.S.C. § 103

Applicants acknowledge the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made. The inventions of each claim of the present application were commonly owned at the time made.

Claim 29 stands rejected under § 103 as being unpatentable over Hunter *et al.* and further in view of Morrison *et al.* The Examiner cites the Morrison reference as teaching the use of EGF to proliferate glial cells. However, as pointed out above, Claim 25, from which Claim 29 depends, concerns the proliferation of neural ***stem cells***. Morrison, citing Almazan *et al.* merely states that "EGF stimulates the proliferation and differentiation of glial cells". The remainder of the Morrison reference goes on to teach that EGF promotes the survival and stimulates process outgrowth of neonatal rat CNS neurons *in vitro*. Process outgrowth is a characteristic of cell differentiation, and promoting survival is not at all the same as proliferation. Thus, Morrison *et al.* do not disclose the proliferation of neural stem cells.

A copy of the Almazan reference, cited by Morrison, is provided with an Information Disclosure Statement filed herewith. Almazan in turn cites references that state that EGF induces proliferation of glial cells (p. 257, col. 1); however, the reference concludes by stating that EGF increases differentiation rather than proliferation (p. 263, col. 2). Thus, even if the reference was concerned with stem cells (rather than progenitor cells or differentiated cells), it would still teach away from using EGF to proliferate stem cells as recited in Claim 29. This, combined with the teaching of Hunter *et al.* that "... epidermal growth factor (EGF) had no growth-promoting effects at the tested doses;" (p. 242, col 1) leads one even further away from the presently claimed invention. Accordingly, even if one were to combine the teachings of Morrison *et al.* and Hunter *et al.*, they would still not arrive at the claimed invention because neither reference discloses a method of producing glial cells derived from neural stem cells.

Claims 1, 3-6, and 35 stand rejected under 35 U.S.C. § 103 as being unpatentable over Boyles *et al.* taken with Hunter *et al.*, Gage *et al.*, and Masters *et al.* Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under §103 requires, inter alia,

consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. In re Vaeck, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

As amended, Claim 1 calls for a method of remyelinating neurons using neural stem cells that produce progeny that are capable of differentiating into neurons, astrocytes, and oligodendrocytes. None of the references cited by the Examiner describe or suggest the proliferation of multipotent neural stem cells in culture to produce precursor cells which are used for remyelination. As discussed above, Hunter *et al.* is concerned with the culturing of progenitor cells rather than stem cells. Similarly, Masters *et al.* only describe the proliferation and differentiation of glial progenitor cells into oligodendrocytes. Boyles *et al.* concerns the identification of apolipoproteins in regenerating peripheral nerves and is not concerned with proliferating neural cell cultures to be used for remyelination. At best, a combination of the references teaches proliferation of progenitor cells. However, the references do not teach the elements defined in steps (a) and (b) of Claim 1, i.e. the isolation of multipotent neural stem cells and the proliferation of these cells in culture.

From a review of the Hall and Watt paper, it can be seen that an advantage of culturing stem cells as opposed to progenitor cells, is that stem cells have the capacity for self renewal (See Fig. 1 of Hall and Watt). This means that a large supply of cells can be cultured from minimal amounts of donor tissue. On the other hand, when progenitor cells are cultured, only limited numbers of cells can be obtained from donor tissue due to limited proliferation of the cells prior to differentiation. Thus, if progenitor cells were to be used commercially where large numbers of cells are required, ethical concerns would exist if the source of the cells is human fetal tissue.

Even if the references were to teach the proliferation of neural stem cells (which they do not), and if the combination of the references were to suggest to one of ordinary skill in the art to try the claimed invention, the prior art provides no reasonable expectation of success that the invention would work as claimed. The Gage reference, relied upon by the Examiner to teach the transplantation of cells to the brain admits the deficiencies inherent in their application:

> "The genetic correction of some, or many, CNS disorders may require the establishment or re-establishment of faithful intercellular synaptic connections. Model systems to study these possibilities have not yet been developed and exploited because of ***the paucity of replicating non-transformed cell-culture systems*** and the refractoriness of non-replicating neuronal cells to viral infection."

(Col 14. lines 61 to 65; Emphasis added). In Example 1 of the Gage reference, the results of astrocyte grafts are described as not having migrated very far from the injection site into the host brain (Col 18. lines 34-39), although optimism is provided that "this apparent lack of migration could certainly be different for other donor cell types" (Col. 18, lines 39 to 44). However, such a statement does not provide one of ordinary skill in the art the requisite reasonable expectation of success that a precursor cell or an oligodendrocyte implanted into a patient will effect the remyelination of a demyelinated axon, as recited in Claims 1 and 7 (and as evidenced by the Reynolds declaration). Indeed, the Examiner alludes to this conclusion on p. 3, lines 18 to 20 of the Office Action, stating that "...in view of the lack of results in the art teaching remyelination via transplantation of cells, it is not apparent that the invention works as claimed".

With regard to Claims 5 and 6, the Examiner states that Gage discloses the transplantation of cells into the human brain and that "it would have been obvious to one of ordinary skill to use the demyelinated axons of the recipient for transplantation

in order to avoid a tissue rejection since Gage discloses that the brain is not a totally immunologically privileged site." To clarify, Claims 5 and 6 do not call for the transplantation of a recipient's demyelinated axon, but rather the isolation of a person's neural stem cells, the proliferation of those stem cells to produce precursor cells, and the transplantation of those cells back into the person. While Gage *et al.* propose that it is desirable to minimize the potential for rejection by using autologous cells wherever feasible, they do not suggest the *in vitro* proliferation of a patient's own neural stem cells and the transplantation of the progeny of the neural stem cells back into the patient. None of the other references cited by the Examiner suggests that this would be possible. Again, the ability to proliferate neural stem cells using applicants' methods allows a large number of cells for transplantation to be obtained from minimal amounts of donor tissue. This is not suggested by Gage *et al.* or the other references cited by the Examiner.

With regard to Claim 4, the Examiner states that "Hunter discloses use of aggregates of cells, also known as neurospheres (page 236, column 2, second full paragraph)". The passage cited by the Examiner describes a method of culturing cells in the presence of high heparin concentration that forces normally adherent cells to form floating aggregates (the term "neurospheres" is not used in the reference). Additionally, the heparin used by Hunter *et al.* aids in obtaining enriched O-2A cultures in that it discourages the growth of type 1 astrocytes. The heparin does nothing to change the potential of O-2A cells which are incapable of differentiating into neurons. The neurospheres defined in applicants' specification contain clonally-derived nestin (+) cells that are non-adherent (p. 8, line 29 to p. 9, line 16). The clonal nature of the cells of a neurosphere is described in more detail above. In contrast, the aggregates described by Hunter are derived from various cells which have migrated together; they are not derived from the proliferation of a single cell. Thus, Hunter *et al.* fail to teach a culture method that utilizes neurospheres to produce clonally-derived progeny as defined by Claim 4.

Claim 2 stands rejected under § 103 as being unpatentable over Boyles taken with Hunter, Masters, Gage and Morrison. The same comments made above with respect to what Morrison *et al.* teach are reasserted here. To summarize, none of the references teach the proliferation of neural stem cells in culture. Rather, at best, they teach the proliferation and survival of progenitor cells. Even if a combination of the references were to teach the proliferation of neural stem cells (which they do not), Morrison *et al.* merely teach that EGF promotes differentiation and survival of cells, not proliferation. Additionally, Hunter *et al.* found no growth-promoting effects when EGF was added to their cell cultures. Thus, contrary to the Examiner's position, one of ordinary skill would not have been motivated to use EGF in the culture method of Hunter in order to obtain large numbers of oligodendrocytes. Even if such motivation were to have existed, it is not relevant because Hunter *et al.* was concerned with the proliferation of glial progenitor cells, whereas the method of Claim 2 calls for the proliferation of neural stem cells.

Claims 7, 8 and 10-17 stand rejected under § 103 as being unpatentable over Boyles taken with Hunter, Masters and Gage. Claim 7 has been amended to recite that the progeny of the neural stem cells of step (a) are capable of differentiating into astrocytes, oligodendrocytes, and neurons. The Examiner states that "Hunter discloses isolation of neural stem cells". However, Hunter only discloses the culturing of glial progenitor cells. The same points raised above with respect to the rejection made to Claims 1, 3-6 and 35 under § 103 with respect to the four references used by the Examiner are reasserted here. A combination of these references would not have provided one of ordinary skill in the art direction on how to proliferate neural stem cells let alone how to use such cells to remyelinate an axon.

Even if a combination of the references were to teach the proliferation of neural stem cells (as opposed to merely glial progenitor cells), they still would not provide the requisite reasonable expectation of success that such cells could effect remyelination as

required by Claim 7. This is because, as raised above, Gage *et al.* were not able to show that implanted cells could migrate very far from the injection site to form cellular connections with host cells.

Regarding Claim 14, the Examiner states that "Hunter discloses that the precursors are in neurospheres". The culture methods of Hunter *et al.* are such that normally adherent cells form floating aggregates due to a high concentration of heparin added to the culture medium. Thus, the aggregates of Hunter *et al.* are not the same as the neurospheres described in the present application which are floating, clonally-derived spheres of cells which form aggregates in the absence of heparin yet in the presence of a substrate onto which normally adherent cells would attach. Claim 14 has been amended to recite that the neurospheres are clonally-derived.

Regarding Claims 15 to 17, the Examiner states that "Gage discloses transplantation of cells to treat diseases of the CNS". However, Gage does not teach or suggest the transplantation of oligodendrocytes that are capable of remyelinating a recipient's axon. Furthermore, Gage *et al.* do not teach or suggest culturing a recipient's own neural stem cells to produce oligodendrocytes for transplantation back into the recipient to effect remyelination of a demyelinated axon. None of the other references cited by the Examiner is able to cure the deficiencies of Gage *et al.* with respect to these elements of Claims 15 to 17.

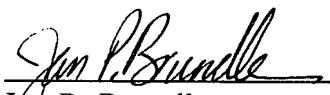
Claim 9 stands rejected under § 103 as being unpatentable over Boyles taken with Hunter, Masters and Gage. The Examiner states that "Morrison discloses that EGF stimulates the proliferation and differentiation of glial cells". However, as noted above, Morrison teaches away from the use of EGF for cellular proliferation and instead teaches the use of EGF for cell survival and differentiation. Additionally, Hunter *et al.* found no growth-promoting effects when EGF was added to their cell cultures. Accordingly, the use of EGF to promote the proliferation of neural stem cells

would not be expected and thus would be unobvious in view of these references that teach away from such use.

In view of the declaration of Joseph P. Hammang, the amendments to the claims, and for the foregoing reasons, it is respectfully submitted that the present claims are in condition for allowance. Early notice of such allowance is solicited.

Respectfully submitted,

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT



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